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Performance of CHROMagar MRSA Medium for Detection of Methicillin-Resistant *Staphylococcus aureus*

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CHROMagar MRSA was evaluated for its ability to identify methicillin-resistant *Staphylococcus aureus* (MRSA). A well-defined collection consisting of 216 MRSA strains and 241 methicillin-susceptible *Staphylococcus aureus* isolates was used. The sensitivity of CHROMagar MRSA after 24 h of incubation was 95.4%, increasing to 100% after 48 h. The specificity was already 100% after 24 h.

Staphylococcus aureus is one of the pathogens most frequently isolated within clinical specimens. In fact, *S. aureus* is currently the most common cause of nosocomial infections (1). Treatment of infections caused by *S. aureus* has become more problematic since the development of antimicrobial resistance. Currently the most important problem is methicillin-resistant *Staphylococcus aureus* (MRSA). Since MRSA strains are resistant to all beta-lactam antibiotics, the therapeutic options are limited significantly. In addition, most MRSA strains are resistant to other groups of antimicrobials. The incidence of nosocomial infections caused by MRSA continues to increase worldwide (16). Screening for MRSA is important for therapeutic and epidemiological reasons. Methods used to detect MRSA in clinical samples ideally should have high sensitivity and specificity and should report the results within a short time. To identify *S. aureus* from contaminated samples more easily and reliably, selective media have been developed. Ideally, selective media achieve isolation of *S. aureus* and detection of methicillin resistance in one step (13).

The purpose of this study is to evaluate the in vitro sensitivity and specificity of a recently developed medium called CHROMagar MRSA, which uses a well-defined collection of strains to identify MRSA.

A well-defined collection of 457 *S. aureus* strains was used. Two hundred forty-one strains were methicillin-susceptible *S. aureus* (MSSA), and 216 strains were MRSA. The isolates were stored at −70°C until they were tested. The 216 MRSA isolates were collected in The Netherlands between 1989 and 1998 and are part of the MRSA strain collection of the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Identification of the strains as *S. aureus* and methicillin resistance were determined by duplex PCR for the *mecA* gene and coagulase gene, as described previously (14). Strains were selected on the basis of their different phage types (12). Bacteriophage typing was performed as described previously (10, 12, 15), by using the inter-

national set of phages at 1 and 100 times the routine test dilution concentrations, an additional set of Dutch phages, and a set of experimental MRSA phages.

The 241 MSSA strains were isolated from cultures of blood collected between May 1996 and June 1999 from patients at the following six Dutch hospitals: St. Elisabeth Hospital and Tweesteden Hospital, Tilburg; Pasteur Hospital, Oosterhout; Tweesteden Hospital, Waalwijk; and St. Ignatius Hospital and Hospital de Baronie, Breda. Only one isolate was included per patient per admission period. Isolates were identified by a latex agglutination test (Staphaurex Plus; Murex Diagnostics Ltd., Dartford, England), by the detection of free coagulase by the tube coagulase test with rabbit plasma (6), and by the detection of DNase (DNase agar; Oxoid Ltd., Basingstoke, England). If the results of these tests were discordant, a culture identification test (Gen-Probe; AccuProbe, San Diego, Calif.) was performed according to the manufacturer's instructions. The result of the AccuProbe test was considered the "gold standard" for the identification of *S. aureus*. The blood culture isolates were classified as methicillin susceptible (MIC of oxacillin, ≤2 μg/ml) at the time of collection by broth microdilution susceptibility testing performed according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS). Furthermore, no growth was observed by the oxacillin agar screen test according to the NCCLS standard (9).

CHROMagar MRSA (CHROMagar Microbiology, Paris, France) is a new chromogenic medium for the identification of MRSA. The composition of the chromogenic and selective mix is proprietary.

The isolates were inoculated on Columbia agar plates with 5% sheep blood and incubated for 24 h at 35°C. From the resulting cultures, a suspension at a 0.5 McFarland standard was made; subsequently, a swab was dipped in the suspension and streaked on a CHROMagar MRSA plate. The results were read after 24 and 48 h of incubation at 35°C. The growth of colonies showing any pink or mauve coloration was considered to be positive (indicating MRSA). The procedure was performed as recommended by the manufacturer.

The results obtained with CHROMagar MRSA are shown in Table 1. A total of 216 MRSA strains were tested. The sensi-

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TABLE 1. Results of CHROMagar MRSA after 24 and 48 h of incubation

Organism	No. of strains with a positive test result/total no. of strains (%) at:	
	24 h	48 h
MSSA	0/241 (0)	0/241 (0)
MRSA	206/216 (95.4)	216/216 (100)

tivity to detect MRSA after 24 h was 95.4% (206 of 216 strains). After 48 h the sensitivity was 100% (216 of 216 strains). A total of 241 MSSA strains were tested. The specificity was 100% after both 24 and 48 h of incubation.

Detection of MRSA from clinical samples is usually accomplished with the use of conventional nonspecific media, such as blood agar. Most of the samples are taken from nonsterile sites, like the nares, throat, perineum, and wounds. Nonspecific media are used to isolate a broad spectrum of microorganisms without inhibiting the growth of MRSA strains for which the oxacillin MIC is low (7). The disadvantage of such media is that confirmatory tests are necessary to differentiate *S. aureus* from other staphylococci. The use of chromogenic media can potentially reduce the number of confirmatory tests and achieve isolation and presumptive identification in a single step (4, 5, 7).

In this in vitro evaluation, CHROMagar MRSA was tested for its capacity to identify MRSA. To screen for MRSA, antibiotic supplements were added to the plates. The sensitivity to detect MRSA after 24 h was 95.4%. Prolonging the incubation period improved the sensitivity to 100% after 48 h of incubation. The specificity was 100% and was not affected by a prolonged incubation period.

In a previous experiment, we evaluated the in vitro sensitivities and specificities of two chromogenic media: oxacillin resistance screening agar base (ORSAB; Oxoid Ltd.) and CHROMagar Staph aureus (CSA; CHROMagar Microbiology). CSA had a significantly higher specificity than ORSAB (7). Only 1.6% of the coagulase-negative staphylococci (CoNS) tested gave false-positive results after 24 h. Prolongation of the incubation period to 48 h increased the percentage of CoNS that gave false-positive results to 6.0%. For screening of MRSA strains, antibiotic supplements were added to both media. The sensitivity was low after 24 h of incubation (CSA, 58.6%; ORSAB, 84.2%) but increased significantly after 48 h of incubation (CSA, 77.5%; ORSAB, 91.4%). The CHROMagar MRSA as used in the present experiment has a significantly higher sensitivity than that determined for CSA or ORSAB in the previous study, which used the same collection, and CHROMagar MRSA can detect MRSA strains for which the oxacillin MIC is low.

Other researchers have studied the clinical performance of selective chromogenic media. Merlino et al. studied a small sample of 136 staphylococci strains: 114 were *S. aureus* and 22 were coagulase-negative staphylococci (8). CSA was compared to conventional mannitol salt agar by using DNase agar plates and the tube coagulase test. The CSA medium was supplemented with methicillin at a concentration of 4.0 mg/liter. None of the MSSA strains grew on the plates, and all 36 highly resistant MRSA strains grew suspect colonies. A collection of

12 community-acquired MRSA strains was also tested. Only four of these 12 strains (33.3%) grew on methicillin-supplemented CSA. Simor et al. compared ORSAB, supplemented with oxacillin, with a conventional mannitol salt agar plate, supplemented with 2.0 mg of oxacillin per liter, for the detection of MRSA in clinical specimens (13). When specimens from patients at high risk for MRSA colonization were screened with ORSAB, 102 of 104 MRSA-positive clinical specimens (98%) were correctly identified. In total, 138 clinical specimens yielded blue, mannitol-fermenting colony types; therefore, the positive predictive value of ORSAB-positive specimens for MRSA was only 74%. Becker et al. (2) reported that the sensitivity of ORSAB was as high as that reported by Simor et al. (13), but positive predictive values were much lower (48.9%) in the study of Becker et al. This discrepancy may be explained by the fact that Becker et al. included all submitted clinical specimens, rather than restricting the experiment to patients at high risk for MRSA colonization. This inclusion will likely result in a lower prevalence of MRSA and, consequently, a lower positive predictive value.

Blanc et al. (3) evaluated the sensitivity of ORSAB medium for the recovery of MRSA from patients' specimens by using ORSAB alone as a primary culture medium and as a subculture of a selective enrichment broth (Mueller-Hinton broth supplemented with NaCl and oxacillin). A low sensitivity (74%) was obtained when ORSAB medium was used alone as a primary culture, whereas the sensitivity was 88% when a single selective enrichment broth was used. The specificity was only 47% in both cases. Blanc et al. concluded that an enrichment broth is still needed to ensure a good sensitivity for the recovery of MRSA. Perry et al. (11) evaluated MRSA ID, a new chromogenic agar medium. Seven hundred forty-seven swabs from various clinical sites were cultured on MRSA ID, CHROMagar MRSA, ORSAB, and selective mannitol broth. A total of 85 MRSA strains were isolated by a combination of all methods. After 48 h of incubation, 89, 72, and 78% of the MRSA strains were isolated on MRSA ID, CHROMagar MRSA, and ORSAB, respectively. There was little difference between CHROMagar MRSA, MRSA ID, and ORSAB for the isolation of MRSA from nasal swabs and axillae after 48 h of incubation. However, CHROMagar MRSA appeared to be inferior to all other media for the isolation of MRSA from perineal swabs, possibly due to the impact of the competing flora.

The present evaluation is different from local studies using consecutive clinical MRSA isolates in an area of endemicity, where only a limited number of clonal types are tested. This circumstance leads to over- or underestimation of the true value of a test under evaluation. In the present study, a specific type was included only once and many different strains were tested. Therefore, this study provides results that represent a broad array of strains.

However, this study has some important limitations. First, a high inoculum of MSSA and MRSA isolates in pure culture was used, in contrast to the low concentration usually present in clinical samples. Second, the presence of CoNS or the influence of other species was not evaluated. This may influence both sensitivity and specificity when CHROMagar MRSA medium is used on clinical samples.

In conclusion, CHROMagar MRSA is highly sensitive and

specific to differentiate between MSSA and MRSA. Since the sensitivity is optimal after 48 h, samples need a minimum of 2 days of incubation before final results can be obtained. Further studies are required to determine the utility of CHROMagar MRSA in detecting MRSA directly from clinical samples.

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